Atty. Docket No: 087714/0113

In re patent application of

SRIKANTHA, THYAGARAJAN *et al.*

Serial No. 09/424,951

Filed: January 20, 2000

For: CANDIDA ALBICANS TWO-COMPONENT HYBRID KINASE GENE, CANIK1, AND USE THEREOF

AMENDMENT IN RESPONSE TO NOTICE UNDER §§1.821-825

Assistant Commissioner for Patents
Washington, D.C. 20231
Box SEQUENCE

Sir:

In response to the Notice to Comply With Requirements for Applications Containing Sequence Disclosures mailed December 15, 2000, please amend the application as follows:

IN THE SPECIFICATION:

Page 3, line ~~11~~ 7, after "sequence" insert --(SEQ ID NO: 1)--;
line ~~13~~ 9, after "sequence" insert --(SEQ ID NO: 2)--;
line ~~17~~ 13, after "sequence" insert --(SEQ ID NO: 3)--;
line ~~18~~ 15, after "sequence" insert --(SEQ ID NO: 4)--.

Page 15, line ~~22~~ 20, after "Slb1:" insert --(SEQ ID NO: 5)--;
line ~~23~~ 22, after "Slb2:" insert --(SEQ ID NO: 6)--;
line 24, after "Slb3:" insert --(SEQ ID NO: 7)--.

REMARKS

Applicants submit this Amendment to insert required references to SEQ ID NOS of the Sequence Listing filed concurrently herewith. Applicants respectfully request examination on the merits of this application.

Respectfully submitted,

15 February 2001
Date

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description. It should be understood, however, that the detailed description and the specific examples, only indicate preferred embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURES 1A-B show (SEQ ID NO:1) ~~FIGURE 1 shows~~ the nucleotide sequence (top row) ~~(SEQ ID NO:1)~~ of the PCR product encoding the region spanning the H1 and D domains and the deduced amino acid sequence of the CaNIK1 protein (SEQ ID NO:2) (bottom row) ~~(SEQ ID NO:2)~~. The amino acid residues of functional domains are underlined. The three degenerate primers used to isolate the PCR products are shown as S1b1, S1b2, and S1b3.

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FIGURES 2A-E show (SEQ ID NO:3) ~~FIGURE 2 shows~~ the nucleotide sequence (top row) ~~(SEQ ID NO:3)~~ of the gene *CaNik1* and the deduced primary amino acid sequence of the CaNIK1 protein (SEQ ID NO:4) (bottom row) ~~(SEQ ID NO:4)~~. The beginning of each unique repeat is represented within the rectangle. The potential amino acid residues of different functional domains are underlined.

FIGURE 3 is a schematic representation of the anatomy of two alleles in two strains of *C. albicans* according to the present invention. All the functional domains are shown as white bold letters inside each rectangle. A few of the unique restriction enzyme sites are shown at the top of the rectangle. The start of the protein coding region is shown as ATG. WO-1 and CAI8 are the two strains analyzed in this invention. H1 and H2 are two identical alleles of the strain WO-1. H1-L and H2-S represent large and small alleles respectively in strain CAI8. The five hatched rectangular units in each allele represent repeat units described in this invention. The gray rectangular area encompassing *Xho*I-*Pst*I in H2-S represents the region containing a deletion of approximately one repeat unit length.

FIGURE 4 illustrates the deletion strategy used to generate a homozygous deletion mutant, HH80, in strain CAI8. The region spanning *Afl*II-*Xho*I was deleted and substituted by a hisG-Urablaster cassette in the plasmid pUNIK12.1 to create pCNH35

phosphorylation and measurement of kinase activity can be found in Chapter 18 of Ausubel *et al.*

In a further example, a reporter gene is operably linked to a promoter and the level of transcription of the reporter gene is monitored after contact between the plurality and the test substance. In accordance with the present invention, the promoter region of the *CaNik1* gene is operably linked to the luciferase gene. Gene activity is thus linked to luciferase activity, which can then be measured quantitively, with a luminometer, as a bioluminescent reaction.

The present invention is described further below by reference to the following examples, which are illustrative only.

Example 1. PCR Amplification to Determine a *CaNik1* Probe

The following, deoxyinosine-containing, degenerate primers were designed that encompassed the highly conserved regions of the two component response regulators *LemA* (Hrabak & Willis, *J Bacteriol* 174: 3011-3020 (1992)), *BarA* (Nagasawa *et al.*, *Escherichia coli*. *Mol Microbiol*, 6: 799-807 (1992)) and *SLN1* (Ota & Varshavasky, *Science* 262: 566-569 (1993)), respectively: 1) ~~(SEQ ID NO:5)~~ (SEQ ID NO:5) S1b1: 5-GAATTGAGAACGCCTITIAATGG-3, which corresponds to the histidine-autokinase domain; 2) ~~(SEQ ID NO:6)~~ (SEQ ID NO:6) S1b2: 5-AGICCTAAGCCA GTACCACC-3, which corresponds to the ATP-binding domain; and 3) ~~(SEQ ID NO:7)~~ (SEQ ID NO:7) S1b3: 5-TTTAGGCATCTGGACITCCAT, which corresponds to the response regulator domain. S1b1 served as a 5'-end primer for PCR amplifications. The S1b1/S1b2 and S1b1/S1b3 pairs were used to amplify PCR products using the Hot-start wax gem (Perkin, Elmer) protocol. The Hot-start wax gem protocol which generates PCR products used the following reaction mixture: 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.2 mM $MgCl_2$, 100 µM dNTP, 50 µm of each primer and 2.5 units of Taq polymerase, in a final volume of 100 µL. Conditions for PCR cycling included denaturation at 94°C for 1 min, annealing at 40°C for 1.5 min and extension at 72°C for 2.5 min. For all amplifications, *S. cerevisiae* genomic DNA was used as a